EFFECTS OF PNEUMOCOCCUS TYPE I ON LEUKOCYTES AND HEMOPOIETIC ORGANS

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THE EFFECT OF PNEUMOCOCCUS TYPE 1 ON THE BLOOD AND HEMOPOIETIC ORGANS OF RABBITS

The blood changes in croupous pneumonia have been much discussed and the occurrence of leukopenia in certain fatal cases has made it doubtful whether negative chemotaxis is the cause of leukopenia in general. Careful study of the blood and hemopoietic organs, which might give fuller information concerning the leukocytic reaction in pneumococcus and other infections, seems indicated.

Halla,[1] Kikodse,[2] and Jaksch[3] all thought that leukopenia in croupous pneumonia is a bad prognostic sign. Cabot[4] reported 6 cases of pneumonia with leukopenia and one case with leukopenia at the beginning and a progressive leukocytosis toward the end of disease, but he does not believe that there is any relation between the number of leukocytes and the severity of disease. According to Ewing,[5] a well marked leukocytosis indicates a severe infection and a leukopenia a bad prognosis. Arneth[6] states that leukopenia with a relative lymphocytosis means in general a severe infection and a doubtful, but not an absolutely bad, prognosis. Williamson[7] calls attention to the fact that a pneumococcus septicemia is usually associated with leukopenia. Rieder[8] found leukopenia with a marked relative increase in the number of polymorphonuclear neutrophils in fatal cases of lobar pneumonia. Limbeck[9] asserts that a fatal case of pneumonia is usually marked by a progressive leukocytosis. Tschistovitch[10] points out the significance of leukopenia by demonstrating experimentally that rabbits inoculated with virulent pneumococci usually die with leukopenia, but in animals inoculated with nonvirulent pneumococcus there is a leukocytosis. Billings[11] states that patients with fatal cases of pneumonia may or may not have leukocytosis, and that a continuous absence of leukocytosis indicates an unfavorable prognosis and high virulence of the pneumococcus.

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1 Ztschr. f. Heilk., 1883, 4, p. 198.

2 Path. Anat. d. Blut. b. Croup Lungenentzündung, Inaug. Diss., 1890.

3 Centralbl. f. klin. Med., 1892, 13, p. 81.

4 Bost. Med. & Surg. Jour., 1894, 130, p. 277.

5 N. Y. Med. Jour., 1893, 58, p. 715.

6 Die Neutrophilon weissen Blutkörporchen, 1904.

7 Zeigler's Beiträge, 1901, 29, p. 41.

8 Beiträge z. Kennt. d. Leukocytose, 1892.

9 Grundriss z. klin. Path. d. Blutes, Jena, 1892.

10 Ann. d. l'Inst. Pasteur, 1891, 5, p. 450.

11 Bull. Johns Hopkins Hosp., 1894, 5, p. 105.

Lambert and Samuels[12] studied the relations between the leukocytes and marrow changes in acute lobar pneumonia, and they found that in some cases there was a parallelism in the number of leukocytes and the degree of the hyperplasia of marrow, in others there was an aplastic marrow with leukocytes well above normal, and in still others there was leukopenia with a hyperplastic marrow. They explain leukocytosis with an aplastic marrow on the basis that there may be a hyperplasia in the bones that may be overlooked and a development of leukocytes in the spleen; but they could not see why there should be a leukopenia with a hyperplastic marrow. Dickson[13] found degenerative changes in the marrow of rabbits with virulent pneumococcus infection. In experimental pneumonia, Welch[14] noted that the changes in spleen and lymph nodes were somewhat dependent on the virulence of the pneumococcus, the virulent strain usually giving rise to deposits of fibrin, hemorrhage, and karyorrhexis and the nonvirulent pneumococcus to a little change or none at all.

In the experiments now reported rabbits were inoculated intravenously or intratracheally with virulent or nonvirulent pneumococcus of type I in 24 hour dextrose-broth cultures. One series of rabbits was inoculated with the filtrate of a very virulent pneumococcus culture, after being passed through a Massen filter.

The tables serve to illustrate the general results of the observations on the leukocytic reaction.

TABLE 1

EXPER. 1, RABBIT A

	Total Number of Leukocytes	Percentage of							
		Amphophils	Basophils	Eosinophils	Lymphocytes	Large Mononuclears	Transitionals	Myelocytes	Degenerate Leukocytes
Before inoculation	8,450	52	4	0	44	0	0	0	0
Days after									
1.....	9,200	50	4	0	42.6	3.4	0	0	0
2.....	12,225	57	4	0	24	9.6	5.4	0	0
3.....	14,475	60.2	2	0	32.2	5.6	0	0	0
4.....	21,250	68.2	3	0	21.6	2.2	5	0	0
5.....	22,850	72.4	3	0	20	0.6	4	0	0
6.....	28,050	69	0.5	0	20.5	4	6	0	0
7.....	27,800	66.2	2	0	12.4	9.4	10	0	0
8.....	25,650	75.4	2	0	10	6	6.6	0	0
9.....	22,725	64.2	1	0	16.8	6	12	0	0

Exper. 1.—Rabbits A and B received intratracheally 2 c c of pneumococcus strain of such virulence that about 0.2 c c of 24-hour broth culture were fatal to a mouse within 4 days. The animals died with marked leukocytosis.

[12] Jour. Infect. Dis., 1918, 23, p. 443.

[13] The Bone-Marrow, 1908.

[14] Bull. Johns Hopkins Hosp., 1892, 3, p. 125.

TABLE 2

EXPER. 1, RABBIT B

	Total Number of Leukocytes	Percentage of							
		Amphophils	Basophils	Eosinophils	Lymphocytes	Large Mononuclears	Transitionals	Myelocytes	Degenerate Leukocytes
Before inoculation	15,625	35.5	3.5	0.5	60	0.5	0	0	0
Days after									
1.....	40,725	84.4	0	0	11	0	4.6	0	0
2.....	28,025	74.2	1	0	13	4	7.8	0	0
3.....	28,375	80.4	2.4	0	10	3.2	4	0	0
4.....	21,275	76	3	0	14	2.4	4.6	0	0
5.....	22,705	79.6	2.2	0.2	10.2	2	7.8	0	12†
6.....	18,700	81.2	0	0	8	4	6.8	0	19.5†

The granules of amphophils appeared brownish and irregular in size.

Exper. 2.—Rabbit B received intravenously 1 c c of virulent pneumococcus, about 0.01 c c being fatal to a mouse within 24 hours. The animal had a leukocytosis about 24 hours after inoculation but died with leukopenia.

TABLE 3

EXPER. 2, RABBIT B

	Total Number of Leukocytes	Percentage of							
		Amphophils	Basophils	Eosinophils	Lymphocytes	Large Mononuclears	Transitionals	Myelocytes	Degenerate Leukocytes
Before inoculation	13,750	45.4	5.2	0	49.4	0	0	0	0
Hrs. after									
4.....	8,350	72.2	4.4	0	16	3.4	4	0	0
24.....	18,750	46	5.4	0	40	5	3.6	0	19
48.....	6,800	49	3.2	0	40	4.8	3	0	23
52.....	4,175	35.4	5	0	45.2	4.4	7	3	22

Exper. 3.—Rabbit A received intravenously 1 c c virulent pneumococcus, about 0.001 c c being fatal to a mouse within 48 hours, The animal died within 48 hours.

TABLE 4

EXPER. 3, RABBIT A

	Total Number of Leukocytes	Percentage of							
		Amphophils	Basophils	Eosinophils	Lymphocytes	Large Mononuclears	Transitionals	Myelocytes	Degenerate Leukocytes
Before inoculation	9,025	31	0.5	0	67.5	1	0	0	0
Hrs. after									
24.....	4,450	48.4	3.2	0	36.4	3	9	0	12
30.....	2,250	51	4	0	34.4	3.6	7	0	All degenerated

Exper. 4.—A series of 3 rabbits received intravenously 3 c c of the cultural filtrate of virulent pneumococcus used in exper. 3. Another rabbit used as control was also inoculated with 3 c c of sterile dextrose-broth. The number of leukocytes was counted every 2 hours within first 8 hours and thereafter every 24 hours for 3 days.

TABLE 5

RESULTS OF EXPER. 4

	Rabbit C	Control Rabbit
Before inoculation	12,450	11,250
2 hours after	8,625	11,450
4 hours after	7,945	9,500
6 hours after	4,500	9,425
8 hours after	3,250	8,225
24 hours after	5,425	10,550
48 hours after	14,500	19,250
72 hours after	12,945	15,500

Exper. 5.—By the smear method I tested the toxic action of a virulent pneumococcus on human leukocytes in vitro. The leukocytic suspension was mixed with various amounts of culture or culture filtrate of the virulent pneumococcus, and the mixtures thus made were then incubated at 37 C. and examined every 15 minutes. The smears were stained with Wright's stain. Table 6 shows the results.

TABLE 6

RESULTS OF EXPER. 5

Leukocytes, C c	Cultural Filtrate, C c	Salt Solution, C c	Results		
			15 Minutes	30 Minutes	45 Minutes
0.1	0.1	0.8	+	+	+
0.1	0.075	0.825	0	+	+
0.1	0.05	0.85	0	±	+
0.1	0.025	0.875	0	0	0
0.1	0.01	0.89	0	0	0
0.1	0.0075	0.8925	0	0	0
0.1	0	0.9	0	0	0
Leukocytes, C c	Pneumococcus Culture, C c	Salt Solution, C c	Results		
			15 Minutes	30 Minutes	45 Minutes
0.1	0.1	0.8	+	+	+
0.1	0.075	0.825	+	+	+
0.1	0.05	0.85	+	+	+
0.1	0.025	0.875	0	+	+
0.1	0.01	0.89	0	+	+
0.1	0.0075	0.8925	0	0	0
0.1	0	0.9	0	0	0

+ = a definite degeneration of leukocytes; ± = doubtful; 0 = negative.

In view of the foregoing results, it is evident that virulence of the pneumococcus has a direct bearing on the leukocytic reaction, a low virulence producing leukocytosis and a high virulence leukopenia. This seems to be in perfect accord with the observations of Tschistovitch and Billings, but it does not explain why there should be leukopenia in

animals infected with a virulent pneumococcus. Bieganski [15] contended that the leukopenia in croupous pneumonia is brought about by the destruction of leukocytes caused by toxic substances produced by the pneumococcus. The filtrate of the very virulent pneumococcus tested in exper. 4 produced an initial leukopenia far more marked than in the control animals injected with broth. This gives rise to an assumption that the leukopenia in exper. 4 as well as in others might be in part due to the destruction of leukocytes caused by the toxic action of the pneumococcus. This is substantiated by the fact that the same filtrate produced a definite degeneration of human leukocytes in vitro. The differential count revealed that the reduction in the number of leukocytes involved a decided gain in the transitional and large mononuclear leukocytes, but the amphophils in the stage of leukopenia showed only a slight deviation from their normal percentage. In no instance were the eosinophils found increased in number.

The morphologic changes of individual cells in the peripheral circulation varied greatly, but in general the changes were practically the same as I have described in the cases of hemolytic streptococcus [16] and diphtheria toxin.[17] However, it should be borne out that the karyorrhexis observed in this study was usually followed by a pyknosis as the nuclear fragments were often seen as black globular bodies of various sizes, and that in the later stages of infection, even with less virulent pneumococci, the granules of amphophils appeared brownish and irregular in size, being in a way similar to the granules described by Alder.[18] In both rabbits in exper. 3 the blood smears made about 4 hours before death showed all the leukocytes in various stages of degeneration and also a considerable number of pneumococci free in the plasma. This seems to illustrate a close relation between the virulence of the pneumococcus and the leukocytic degeneration. The morphologic changes is leukocytes in vitro consisted of pyknosis and an increase in the acidophilia of the cytoplasm of polymorphonuclear neutrophils. The lymphocytes and other forms were less affected. The changes occurred much earlier and were more marked with the whole culture than with the filtrate.

The main changes observed in the marrow in the rabbits experimented on was hyperplasia of the leukoblastic cells which was less

[15] Deutsch. Arch. f. klin. Med., 1894, 53, p. 433.
[16] Jour. Infect. Dis., 1921, 29, p. 141.
[17] Ibid., p. 408.
[18] Schweiz. med. Wchnschr., 1921, 51, p. 437.

marked in the case of infection with virulent pneumococci. In addition to this, the virulent pneumococci also produced localized or generalized degenerative changes (Fig. 1) characterized by karyorrhexis, karyolysis, vacuolation and alteration of staining property, but cultural filtrates did not have any definite injurious effects in this respect. In one rabbit there was a leukopenia preceded by a moderate leukocytosis, the marrow showing a hyperplasia but no degenerative changes. This may be attributed to the fact that the destruction of the leukocytes in the peripheral circulation may be far beyond the power of reparation by the marrow. The sections of marrow from the rabbits in exper. 3

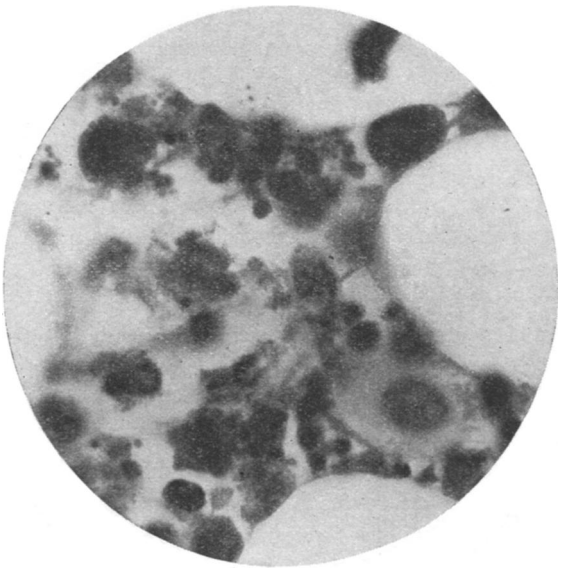

Fig. 1.—Marrow of rabbit in exper. 3; karyorrhexis of myelocytes; × 1000.

revealed, besides a very extensive degeneration, a considerable number of pneumococci. Congestion and hemorrhage were frequently encountered, but the latter was more extensive in acute cases. Megalokaryocytes often occurred in large numbers and none appeared normal.

The spleen was usually more or less degenerated and congested. The malpighian bodies were reduced in size and usually found in areas that contained fibrin, erythrocytes and nuclear fragments. In exper. 1, rabbit B, there was a marked proliferation of phagocytic cells which were crowded with degenerated erythrocytes. The lymph-nodes did not reveal many changes except congestion and sometimes distention of sinuses.

THE BEHAVIOR OF GUINEA-PIG LEUKOCYTES TOWARD PNEUMOCOCCUS TYPE 1

In spite of the great amount of work on phagocytosis, the failure of leukocytes to take up virulent bacteria remains an unsolved problem.

Virulent bacteria, as Massart[19] states, repel leukocytes, a property which is independent of toxin action. He demonstrated that leukocytes manifested a phagocytic activity toward diphtheria bacilli and hog-cholera bacilli, but were inactive in the presence of virulent anthrax bacilli. According to Bordet,[20] leukocytes have a selective action on certain bacteria, guided by chemotaxis, as shown by the fact that the same leukocytes would ingest proteus vulgaris but not virulent streptococci. He states that virulent streptococci do not paralyze

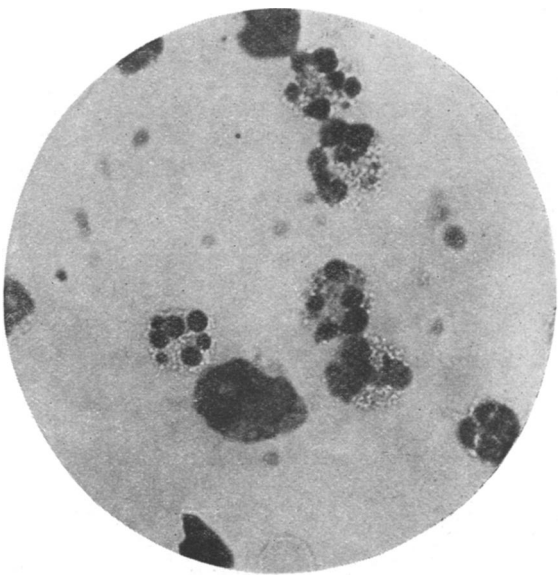

Fig. 2.—Fragmentation of nuclei of amphophils with a marked condensation of chromatin. The granules are highly refractive.

the leukocytes as the latter made ameboid movements in the presence of virulent streptococci. This is confirmed by Matalnikow[21] who found that phagocytes after coming in contact with virulent pneumococci were still able to take up carmine particles. On the contrary, Werigo[22] doubts whether virulent bacteria exert a negative chemotaxis on leukocytes as he found that even virulent anthrax bacilli were susceptible to phagocytosis.

In my experiments, guinea-pigs were inoculated intraperitoneally with virulent and nonvirulent pneumococci of type 1, and the exudate

[19] Ann. d. l'Inst. Pasteur, 1892, 6, p. 321.
[20] Ibid., 1896, 10, p. 104.
[21] Ibid., 1921, 35, p. 363.
[22] Ibid., 1894, 8, p. 1.

withdrawn at intervals. In case of a thick exudate dilution with salt solution was made in order to obtain thin smears. The smears were stained with the Wright, the Gram and the Welch capsule stains.

In the animals inoculated with less virulent pneumococci, 0.1 c c of an 18-hour broth culture being fatal to a mouse within 24 hours, there was usually a marked ingestion of pneumococci by the leukocytes. About 16 hours after inoculation the leukocytes began to undergo

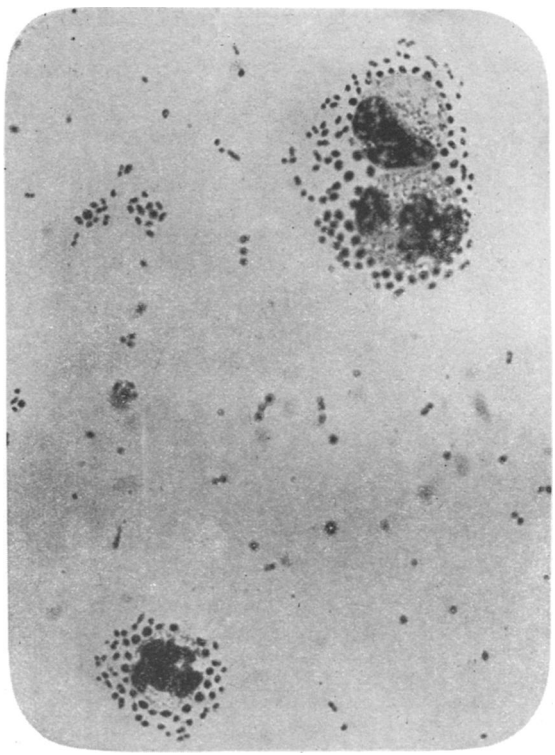

Fig. 3.—The leukocytes are surrounded by large number of pneumococci; two leukocytes are swollen and their granules have lost their refractive property and are stained dark brown with Wright stain so that they appear as cocci.

karyorrhexis and occasionally pyknosis (Fig. 2). In the presence of virulent pneumococci, 0.01 c c of an 18-hour broth culture being fatal to a mouse within 24 hours, there was little phagocytosis throughout the course of the infection, and the leukocytes soon became swollen and surrounded by pneumococci (Fig. 3.). In one case, the leukocytes obtained about 2 hours before death were surrounded by large numbers

of pneumococci arranged in rows, the cocci nearest to the leukocytes undergoing involutional changes as their size had increased. Rosenow [23] found that if leukocytes were heated at 60 C. for 5 minutes, they lost their phagocytic activity but became surrounded by pneumococci, apparently by virtue of chemotaxis. In my experiments, the degenerative changes in the leukocytes seem to indicate an extreme intoxication; in the second place, the involution of the pneumococci themselves suggests the idea that the leukocytes secrete a ferment of unknown nature which is detrimental to pneumococci. One hour after inoculation with highly virulent pneumococci, 0.001 c c of an 18-hour broth culture being fatal to a mouse within 24 hours, the peritoneal exudate consisted of a few amphophils which were inactive toward the pneumococci; about 3 hours later the pneumococci and amphophils both had increased in number, but the latter presented ill-defined outlines with a swollen nucleus in most instances. Only a few leukocytes showed a little phagocytosis. There were also large numbers of so-called leukocytic shadows. If the animals remained alive longer, say 24 hours, the peritoneal exudate consisted largely of mature amphophils which were undergoing degeneration, without any indications of phagocytosis.

In an atempt to corroborate the results of Bordet and Metalnikow, I inoculated a guinea-pig with highly virulent pneumococci and 14 hours later the peritoneal exudate was withdrawn for testing the phagocytic activity of the leukocytes in vitro. To a small amount of exudate, previously diluted with citrate solution, 0.1 c c of pneumococcus was added. The mixture was then incubated at 36 C. and examined every 15 minutes for one hour. At the same time the animal was inoculated again with 1 c c of B. coli in order to observe the behavior of leukocytes toward the bacilli in the presence of virulent pneumococci. It was found that about 2 hours after inoculation with B. coli, a few leukocytes began to take up a few bacilli, but in vitro the leukocytes showed no phagocytic activity. This suggests that the leukocytes that ingested B. coli in vivo might be cells which migrated into the peritoneal cavity after inoculation with B. coli and took up B. coli before becoming intoxicated. If this supposition is true, why are the pneumococci not subjected to phagocytosis? This can be explained only by a selective action of leukocytes as Bordet advocated.

[23] Jour. Infect. Dis., 1906, 3, p. 683.

SUMMARY

The leukocytic reaction in rabbits infected with pneumococcus type 1 is somewhat dependent on the virulence of the organisms, a low virulence producing leukocytosis and high virulence leukopenia.

The leukopenia seems to be brought about by degeneration of leukocytes and of cells in the hemopoietic organs. This degeneration seems to be due to the toxic action of the pneumococcus.

After an intraperitoneal inoculation of guinea-pigs with virulent and nonvirulent pneumococci, the leukocytes as a rule show phagocystosis. In certain instances the failure of leukocytes to take up highly virulent pneumococci seems to be due to intoxication of leukocytes as evidenced by degenerative changes.

It seems that virulent pneumococci also produce a chemotactic substance, as although the leukocytes may fail to ingest virulent cocci they usually become surrounded by them.